Larval Morphometrics and Influence of Adults on Settlement in the Gregarious Ophiuroid *Ophiothrix fragilis* (Echinodermata)

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Abstract. The development of *Ophiothrix fragilis* was documented using light microscopy, and the allometry of larval growth was quantified. Larval development to the suspended juvenile stage took 21 days under conditions that were probably optimal compared to those in the plankton. Larval shape changed through development as the larval body and arms grew. Growth of the posterolateral larval arms was continuous throughout development, even during metamorphosis when the larva became endotrophic. During this period, these larval arms function as locomotory organs, and their continuous growth is probably essential to support the juvenile as it increases in density through development of its calcareous plates. In induction assays using adult conspecifics, initiation of metamorphosis was spontaneous. Release of the posterolateral arms was induced by the presence of adults. This response is likely to enhance a juvenile's chance of recruiting to a suitable habitat in the *Ophiothrix fragilis* beds of the North Sea.

Introduction

Planktotrophic larvae of marine benthic invertebrates face two sets of challenges during development (McEdward and Herrera, 1999). Firstly, they must function as feeding planktonic organisms which must acquire food, swim, control their position in the water column, and select a microhabitat. Secondly, they must undergo ontogenetic transformations that result in continuous changes in body form. This is especially true in the later stages of development when

the rudiment of the juvenile is constructed in preparation for settlement. As a consequence, functional requirements and ontogenic changes can impose demands on larval design (McEdward, 1984, 1986; McEdward and Herrera, 1999). Therefore, the analysis of the development of feeding larvae requires an understanding of the requirements and capabilities of particular stages as well as an understanding of the patterns of larval growth that define the developmental trajectory (McEdward and Herrera, 1999).

Planktotrophic plutei of ophiuroids can develop *via* two pathways depending on their metamorphosis, which occurs in the plankton and precedes settlement (Mladenov, 1985a). In type I developers, metamorphosis is characterized by the regression of all larval arms except for the posterolateral pair and the differentiation of a juvenile between these. In type II developers, all larval arms regress and a vitellaria that differentiates into a juvenile is formed. The larval development of type I developers has been described in detail for four species (MacBride, 1907; Narasimhamurti, 1933; Olsen, 1942; Tominaga *et al.*, 2004).

The brittle star *Ophiothrix fragilis* (Abildgaard, 1789) occurs in high densities throughout the English Channel-North Sea region where the major populations are known and have been studied throughout the late 19th and 20th centuries (Allen, 1899; Warner, 1970; Holme, 1984; Davoult *et al.*, 1990). These populations are considered stable through time and space. *O. fragilis* is a type I planktotrophic developer whose development was described by MacBride (1907). The fact that in the English Channel-North Sea region juveniles are preferentially found on adult conspecifics has led many to believe that settlement occurs preferentially on adults (Warner, 1971; Davoult *et al.*, 1990; Morgan and Jangoux, 2004). Morgan and Jangoux (2004)

have also shown the close relationship between juveniles and adults in this species; this relationship is particularly evident in the way juveniles respond to adults at a distance.

In this study, the development and growth of the larval body and skeleton of *O. fragilis* was followed to document the larval morphometric changes. Considering the well-known aggregative behavior of *O. fragilis*, the role that conspecific adults may play in induction of settlement was investigated.

Materials and Methods

In 1999, specimens of *Ophiothrix fragilis* were collected by scuba diving (20–25 m) from Wemeldinge (Zeeland, Netherlands). Individuals were taken during the period of sexual maturity between the months of May and August and brought back to the Marine Biology Laboratory in Brussels. They were kept in a closed-circuit aquarium under the same abiotic conditions of temperature and salinity as those found in the field (16–18 °C, 32‰).

Fertilizations assays were made using ripe individuals. Individuals were considered ripe when their gonads (white testes; orange ovaries) could be seen through the extended wall of the bursae. Water used for fertilizations and subsequent larval cultures came from the sampling site. Water was allowed to settle for 48 h, then filtered (15 µm) before use.

In each fertilization assay, one male and one female were placed in a small aquarium with 500 ml of filtered and aerated seawater (16 °C, 32‰). The male was agitated for 5 s every minute until it started to liberate its gametes. Release of sperm induced the female to spawn, and the male was removed (Morgan and Jangoux, 2002). Embryos at the gastrula stage were transferred to 30-l larval rearing tanks at a density of 500 embryos/liter.

Larvae from a single spawning event were reared under a 12:12 dark/light photoperiod at 16 °C and 32‰ salinity. Seawater was constantly aerated, and half the volume of water from each tank was siphoned off every 5 days and replaced with filtered seawater. At the onset of larval exotrophy (3rd day after fertilization) the larvae were fed the diatom *Chaetoceros calcitrans* at a concentration of 30,000 cells · larva^{-1} · day^{-1}.

The larvae from this culture were followed from fertilization to completion of development (settled juveniles). Every 1–2 days, a subsample of 30 to 50 larvae was photographed, using a digital camera mounted on a dissecting microscope. Measurements were then made using an image analysis program (UTHSCSA image tool developed at the University of Texas Health Science Center at San Antonio, Texas, and available from the Internet through http://ddsdx.uthscsa.edu/dig/itdesc.html). Two measurements were taken on each individual: the height of the body (body length) and the length of the longest posterolateral rod (Fig. 1).

To establish the chronology of development and morphological change, the larvae were observed every 1–2 days under a microscope with or without polarized light (observation of the skeleton). The timing of development or regression of various features (*e.g.,* larval arms, juvenile mouth) was determined and used as a quantifiable character when at least 50% of the observed larvae had acquired the new feature.

Late larvae from a culture (*n* = 1) were used to test for induction of metamorphosis and settlement. They were placed in a small aquaria filled with 500 ml of filtered autoclaved seawater with (experimental) or without (control) an adult conspecific. In total, 90 larvae divided into three trials were used for each experimental and control assay. The larvae were separated from the adult by a 90-µm mesh. In the case of metamorphosis induction assays, the larvae were observed for 48 h to check whether adult conspecifics could initiate or accelerate metamorphosis. In the case of settlement induction assays, the number of settled individuals was counted after 12 h in experimental and control batches.
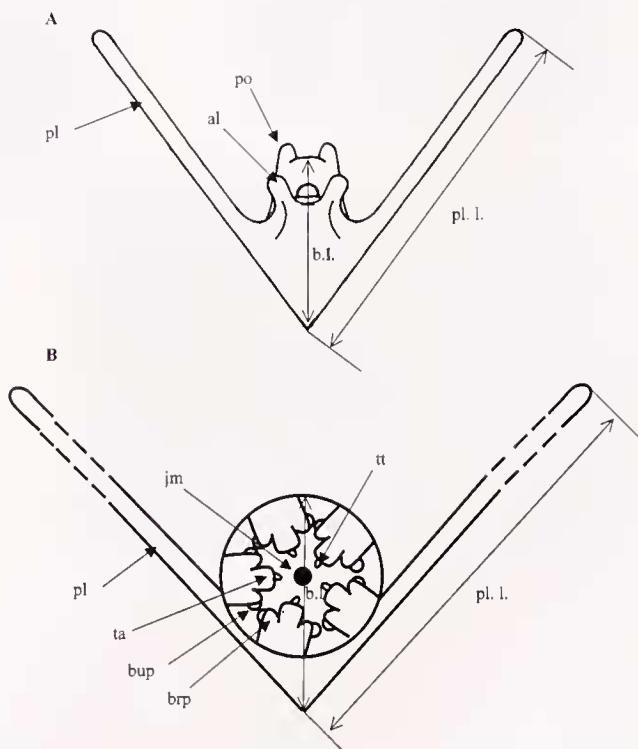

Figure 1. *Ophiothrix fragilis.* Drawing of a 5-day-old larva (A) and a 21-day-old suspended juvenile (B), showing the measurements taken (not to scale). b.l.: body length; pl. l.: posterolateral rod length; al: anterolateral arms; brp: brachial podia; bup: buccal podia; jm: juvenile mouth; pl: posterolateral arm; po: postoral arms; ta: terminal article; tt: terminal tentacle.

Results

Larval development

Table 1 gives the chronology of development of *Ophiothrix fragilis* from fertilization through appearance of settled juveniles. Under our rearing conditions, development was completed in about 21 days. A well-formed oval larva with a vacuolated crest appeared 12 h after fertilization (Fig. 2A). The first pair of arms, the postero-lateral ones, appeared after 24 h (Fig. 2B), and on the 3rd day the openings of the mouth and anus marked the start of the exotrophic larval period. The following days were essentially characterized by the acquisition of additional arms : the anterolateral, postoral and postdorsal pair (Fig. 2D–G). From the 13th day, the left hydrocoele, visible by transparency, started to differentiate five lobes whose development was completed 2 days later, marking the onset of metamorphosis (Fig. 2H). Metamorphosis was initiated when the complete five-lobed hydrocoele began to encircle the esophagus and the so-called ophiurid rudiment began to appear. The larval arms, except the posterolaterals and their respective skeletal rods, started to regress (Fig. 2I, J). Rod regression occurred parallel to the development of juvenile skeletal plates (Fig. 2J). The larval esophagus and intestine regressed in turn, and the larva then entered an endotrophic period, being deprived of both mouth and anus. While endotrophic, the larva progressively became pentameric with the appearance at the rudiment level of radial and interradial areas and the differentiation of five (radial) terminal articles and tentacles. The opening of the juvenile mouth soon followed, and the rudiment continued to develop; in each radius a first pair of ambulacral podia and a pair of buccal podia formed (Fig. 2K, and Fig. 1B for schematic representation). Twenty-one days after fertilization, the resulting suspended juveniles had five podia in each radius and tended to sink to the bottom of the rearing tanks. This event was soon followed by the loss of the remaining pair of larval arms and settlement of the juveniles on the bottom of the tanks. Settled juveniles have a pair of hooked spines on the penultimate arm segment of each radius (see Morgan and Jangoux, 2004).

Larval growth

The length of the longest posterolateral rod, the body length, and their ratio were followed during development. Growth of the posterolateral rod was accelerated during the first couple of days following the embryonic period (Fig. 3A). Growth then slowed down, with a gain of about 300 μm (mean: 279.04 μm; SD: 71.59 μm) between the 3rd and 11th day; it then picked up again, with a gain of 900 μm (mean: 887.91 μm; SD: 111.06 μm) between the 11th and 19th day (Fig. 3A). The posterolateral rod did not stop growing during metamorphosis, from the 16th day onward, but slowed down once it had ended.

Growth of the body was slow during the first couple of days following the embryonic period (Fig. 3B). A slow but constant increase in body length then took place, with a gain of 100 μm (mean: 103.07 μm; SD: 17.51 μm) between the 3rd and 13th day (Fig. 3B). During the differentiation of the five lobes of the left hydrocoele, growth of the body increased. The larval body grew by 70 μm (mean: 72.1 μm; SD: 25.79 μm) in 2 days. Formation of the rudiment provoked a reorganization of the body, with a decrease of 50 μm (mean: 49.47 μm; SD: 23.5 μm).

During the embryonic period, preferential growth of the posterolateral rods took place and the arm-to-body ratio increased sharply (from 1.2 to 2.8), showing allometric growth (Fig. 3C). Growth then became isometric, with the arm-to-body ratio stabilizing around 3 until the 11th day (Fig. 3C). At that point the ratio increased once again showing preferential growth of the arms; by the 15th day, at the onset of metamorphosis, the ratio exceeded 4. During metamorphosis, the decrease in the length of the body and

Table 1

Ophiothrix fragilis: *Chronology of major developmental stages and events*

| Developmental stages and events	Time after fertilization (days)	
	Present study	McBride (1907)
Gastrula	0.5	0.5
2-arm endotrophic pluteus	1	1
4-arm exotrophic pluteus	3	3
6-arm exotrophic pluteus	3	4
8-arm exotrophic pluteus	7	7–10
Fully developed 8-arm pluteus	12	ND
Development of 5 lobes of hydrocoele	13	ND
5-lobed hydrocoele complete	16	16–20
Initiation of metamorphosis	16	20–23
Regression of larval mouth and anus	18	ND
Development of terminal tentacles	18	ND
Development of brachial and buccal podia	19	ND
Apparition of juvenile mouth	19	ND
Suspended juvenile (settlement stage)	21	ND
Benthic juvenile (with hooked spines)	22	26

ND, not determined.
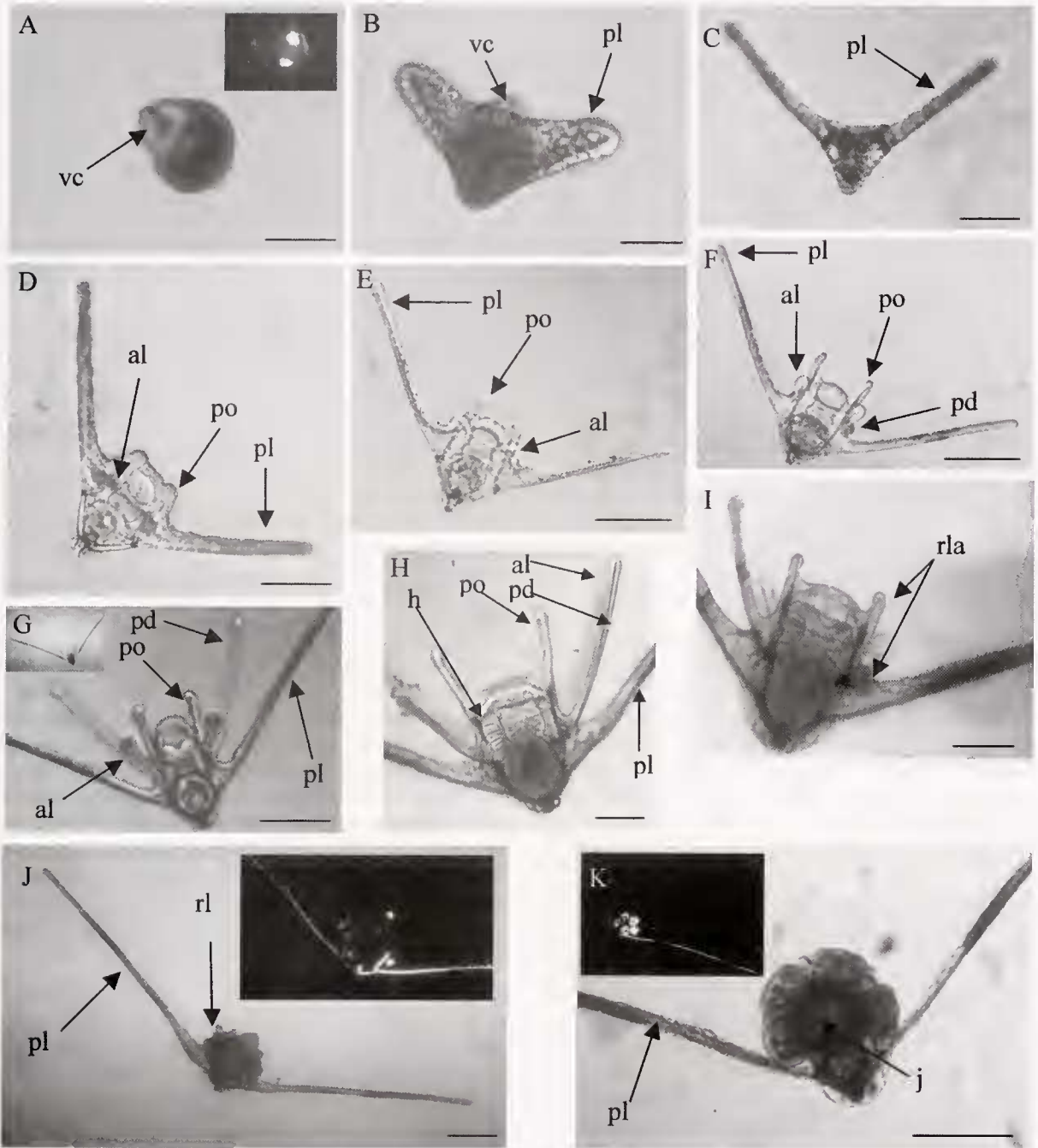
Figure 2. *Ophiothrix fragilis.* Larval development: (A) 12-hour-old larva (inset shows developing larval skeleton); (B) 24-hour-old larva (2-arm endotrophic pluteus); (C) 2-day-old larva (2-arm endotrophic pluteus); (D) 3-day-old larva (6-arm exotrophic pluteus); (E) 5-day-old larva (6-arm exotrophic pluteus); (F) 7-day-old larva (8-arm exotrophic pluteus); (G) 11-day-old larva (inset shows a general view of the 8-arm exotrophic pluteus); (H) 15-day-old larva; (I) 16-day-old larva (metamorphic larva); (J) 19-day-old individual (suspended prejuvenile) (inset shows the regression of the larval skeleton [except for the posterolateral rods] and the developing juvenile skeletal plates); (K) 21-day-old individual at the settlement stage (inset shows the complete juvenile skeleton and the remaining posterolateral rods). al: anterolateral arms: h: hydrocoele; j: juvenile; pd: postdorsal arms; pl: posterolateral arms; po: postoral arms; rla: regressing larval arms (also rl in part J); vc: vacuolated crest. Scale bars: 50 μm (A); 100 μm (B, C, F, G, H, I); 200 μm (D, J, K).
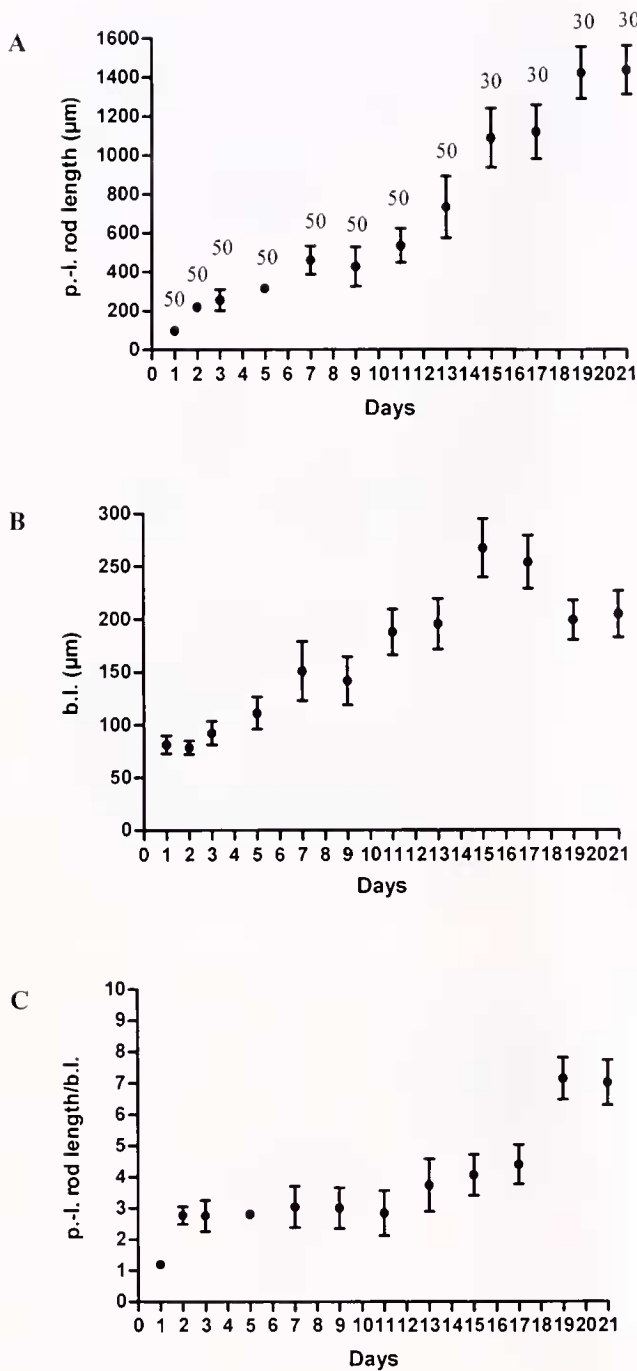
Figure 3.   *Ophiothrix fragilis.* (A) Posterolateral rod growth, (B) body length growth, and (C) ratio of posterolateral rod length to body length during development. b.l.: body length; p.-l. rod length: posterolateral rod length. Values of *n* (number of individuals measured) for A to C are indicated in A. Bars are standard deviations.

increase in the length of the posterolateral rod further accentuated the arm-to-body ratio, which increased from 4 to 7. The arm-to-body ratio stabilized once metamorphosis was complete (Fig. 3C).

Induction of metamorphosis and settlement

The influence of the presence of adults on induction of metamorphosis and settlement was examined at the onset of metamorphosis (larva with a 5-lobed hydrocoele) and the onset of settlement (suspended juveniles), respectively. The presence (experimental) or absence (control) of adult conspecifics did not influence the initiation of metamorphosis: all individuals ($n = 180$) spontaneously began metamorphosis in the water column. The presence of adult conspecifics did not accelerate metamorphosis: all individuals in the presence of adults (three replicates of 30 larvae) were at the same stage of development as the controls (three replicates of 30 larvae). In the settlement induction assays, about 10% (mean: 11.1% in three replicates of 30 larvae) of suspended juveniles settled spontaneously in the controls; in the presence of adults, 90% (mean: 90% in three replicates of 30 larvae) of suspended juveniles settled.

Discussion

In our rearing conditions, *Ophiothrix fragilis* completed development in 21 days. In MacBride's (1907) study, development took 26 days (Table 1). The difference is probably due to larval nutrition. As a food supply, MacBride (1907) used seawater from the field, which is usually a poorer food source than a laboratory ration enhanced with cultured food. (Paulay *et al.,* 1985; Strathmann *et al.,* 1992).

Growth of the larva of *O. fragilis* is continuous throughout its development and can be divided into three periods. During the first period, when the larva is endotrophic, we observed a faster growth of the posterolateral rods compared to body length. This could be due to development of the feeding organs (ciliated band) before the larva becomes exotrophic. The brief endotrophic period during the first days following fertilization and the faster development of the feeding organs during this time also occurs in other echinoderms (McEdward, 1984; Chia and Walker, 1991; Pearse and Cameron, 1991). The second period is essentially characterized by the acquisition and growth of the three other pairs of larval arms, along with growth of the posterolateral rod and growth in body length so that their ratio remains stable (isometric growth). During the third period, the body and posterolateral rod continue to lengthen, with conspicuous development of the arms. Unfortunately, morphometric comparisons can be done with plutei from echinoids only. McEdward (1984, 1986) has shown in different species of sea urchins an initial rapid increase of the whole larval length (which can be equated to our posterolateral rod length). This is followed by a decrease in the rate of elongation during differentiation of further larval arms and, finally, by an increase in the rate of elongation during rudiment development. McEdward suggested that the addition of new arms and their growth during the later stage helps maintain feeding capability relative to larval body size

and metabolism, which also increase during development, especially during rudiment formation. While this could be what happens in echinoplutei, which do not lose their ability to feed during rudiment differentiation, in ophioplutei the future juvenile (or rudiment) is formed from the larval body and the larva passes through a second endotrophic period during which the juvenile digestive tract differentiates. Therefore, the posterolateral arms would essentially be used as locomotory organs and not as feeding-locomotory organs as in echinoids (McEdward, 1984, 1986). As the juvenile differentiates so do its skeletal plates, which would probably increase its weight and make it sink. Although active movement downward probably also occurs, the increase in growth rate of the posterolateral rods could help compensate for the reduction of the other larval arms during metamorphosis, thus allowing the developing juvenile to remain in the plankton.

Metamorphosis in type I developers has been studied in the past by MacBride (1907) on *Ophiothrix fragilis*, Narasimhamurti (1933) on *Ophiocoma nigra*, Olsen (1942) on *Ophiopholis aculeate*, and Tominaga *et al.* (2004) on *Ophiodapline formata*. As numerous authors have already established (Burke, 1989; Hendler, 1991; Byrne and Selvakuramaswamy, 2002), metamorphosis begins spontaneously in the water column when the five-lobed hydrocoele starts to encircle the esophagus and the larval arms begin to regress, and ends with the loss of the two remaining posterolateral arms. Therefore, ending of metamorphosis in ophiuroids coincides with the settlement of the fully functional juvenile. Accordingly, a perimetamorphic period, as defined by Gosselin and Jangoux (1998) for echinoids, can be determined. In echinoids the perimetamorphic period starts at larval competence, includes metamorphosis and the endotrophic postlarval development, and stops at the acquisition of juvenile exotrophy. In ophiuroids the perimetamorphic period starts with the initiation of metamorphosis and ends with the settlement of an exotrophic juvenile. So metamorphosis in echinoids is rapid, but the benthic postlarva still needs to develop to become exotrophic; metamorphosis in ophiuroids is gradual, but it ends with a benthic exotrophic juvenile (juvenile mouth open; see Morgan and Jangoux, 2004). We see that, although the pathways to the exotrophic juvenile are different, both rely on a rapid establishment in the benthos.

Although delay of metamorphosis or settlement is known to occur in a number of marine invertebrates (Highsmith and Emlet, 1986; Pechenik, 1990; Vaitilingon *et al.*, 2001), it is not known if it takes place in ophiuroids and, if it does, upon which developmental stage it operates. Although McBride (1907) described a five-lobed hydrocoele appearing after 16–20 days of development in *O. fragilis*, encirclement of the esophagus, and therefore metamorphosis, did not occur until a few days later. This does not fit our observations, in which the encirclement immediately followed the appearance of the five-lobed hydrocoele. Delay could therefore have occurred, not due to the absence of an inducer as in echinoids—since in ophiuroids metamorphosis begins spontaneously—but due to some other factor, such as a shortage of food resources. In type II developers, delayed metamorphosis is also known to occur at this stage (Mladenov, 1985b). Furthermore, Turon *et al.* (2000, p. 204, fig. 1) illustrated a suspended juvenile of *O. fragilis* bearing two extra articles on each arm. Our results have shown that suspended juveniles can settle at a much earlier stage, indicating a probable delay of settlement in Turon's specimen. Delayed settlement has been observed in other species, and some authors consider the suspended juvenile to be an exploratory stage, as the vittelaria is considered to be in type II developers (Stancyk, 1973; Strathmann, 1978; Mladenov, 1979, 1985b). This interpretation would indicate a probable selection of settlement site.

The larvae of many species settle and metamorphose selectively on substrata with specific physical or biological characteristics (Scheltema, 1974; Crisp, 1976; Burke, 1986). Juveniles of *O. fragilis* in the North Sea-English Channel region have been exclusively found on conspecific adults (Warner, 1971; Davoult *et al.*, 1990; Morgan and Jangoux, 2004), whereas juveniles of their Mediterranean counterparts are found gregariously on sponges before moving to the adult habitat where, once growth has taken place, they live solitarily (Turon *et al.*, 2000). This has led many authors to think that the feeding capabilities of juveniles in the first stages of benthic life would need to be enhanced by the suspension-feeding activities of other organisms (Warner, 1971; Turon *et al.*, 2000, Morgan and Jangoux, 2004). Survival of the juveniles therefore depends on finding a suitable settlement site.

In ophiuroids, metamorphosis begins spontaneously in the plankton. However, loss of the two posterolateral rods and settlement, although it can also happen spontaneously, is clearly enhanced in *O. fragilis* by the presence of adult conspecifics. This is the first case in which an actual induction of settlement has been revealed in ophiuroids. Contact between the settlement stage and the adult is not necessary to induce the breakage of the posterolateral rods. Loss of their posterolateral rods in proximity to an *Ophiothrix fragilis* bed would ensure that the juveniles settle on a suitable site. Furthermore, the hooked spines on juveniles that have lost their posterolateral rods would help them cling to the adults (Morgan and Jangoux, 2004). While our experimental setup has shown that the proximity of an adult conspecific can induce settlement, we still do not know whether juveniles at the settlement stage need to be close to a conspecific adult or can detect them from farther away. MacBride (1907) noted that when larvae in the plankton were reaching the end of their metamorphosis, they sank lower in the water. We also observed this in our cultures.

Juveniles that lose their posterolateral rods spontaneously

will rain down on the available substrata. If it is unsuitable, heavy mortalities would supposedly occur. However, resuspension, drifting, and recolonization following settlement has been demonstrated in some ophiuroids (Hendler *et al.*, 1999). Turon *et al.* (2000) have postulated that recolonization through resuspension could contribute to the mass recruitment of *O. fragilis* juveniles on sponges in the Mediterranean. It could also be that suspended juveniles are induced to settle by some cue released by the sponges, and further studies need to be made along that line.

Induction of loss of the two posterolateral rods and settlement implies the recognition of an inducer. In echinoids, the primary podia are used to test the substrata before metamorphosis (Burke, 1980; Gosselin and Jangoux, 1998). Nothing is known of the sensory structures involved during settlement in ophiuroids, but Morgan and Jangoux (2004) have demonstrated the role that the terminal tentacles of settled juveniles of *O. fragilis* play in the recognition of adult conspecifics at a distance. These organs are already present in suspended juveniles and could therefore be implicated in their recognition of adult conspecifics and in the induction of settlement.

Acknowledgments

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